

SYNERGISTIC EFFECT OF SOME ADDITIVES ON THE BIOLOGICAL ACTIVITY AND TOXICITY OF NEEM-BASED FORMULATIONS AGAINST THE COWPEA APHID, *APHIS CRACCIVORA* KOCH

NADIA Z. DIMETRY AND FATMA M. A. EL-HAWARY

Department of Pests and Plant Protection, National Research Centre, Dokki, Cairo, Egypt

(Accepted 1 December 1997)

Abstract—The effects of various concentrations of Neem Azal-T and Neem Azal-T/S as natural insecticides on the biology and percentage mortality of adult *Aphis craccivora* Koch were studied under laboratory conditions. Both extracts had an aphicidal effect against the adults and significantly decreased their fecundities and longevities. Addition of synergists such as ethyl oleate, sesame oil and dimethyl sulphoxide (DMSO) improved the aphicidal effects of both insecticides. The biological activity of Neem Azal-T/S only could be enhanced with suitable additives as they adversely affected the fecundity and longevity in comparison to the extract alone. Addition of CaCO_3 to the above extracts had no clear effect on either neem seed extract formulation.

Key Words: additives, *Aphis craccivora* Koch, neem seed extract formulations

Résumé—Les effets de différentes concentrations de Neem Azal-T et Neem Azal-T/S, produits utilisés comme insecticides naturels agissant sur la biologie et le taux de mortalité des adultes de *Aphis craccivora* Koch ont été étudiés dans les conditions de laboratoire. Les deux extraits ont montré un effet aphicide contre les adultes et ont réduit leur fécondité et leur longévité d'une façon significative. L'incorporation des matières synergiques comme l'éthyle-oléate, l'huile de sésame et le diméthyle-sulphoxide (DMSO) a amélioré l'effet aphicide des deux insecticides. L'activité biologique de Neem Azal-T/S pouvait seulement être augmenté moyennant l'incorporation des adjuvants appropriés et ceci réduisait la fécondité et la longévité, en comparaison avec l'utilisation de l'extrait brut seul. L'addition du CaO_3 à ces deux produits n'a pas montré d'effet apparent sur la formulation des extraits obtenus à partir de graines de nim.

Mots Clés: additifs, *Aphis craccivora* Koch, formulations des extraits de grains de nim

INTRODUCTION

Aphids are pests of worldwide importance and cause heavy crop losses either by feeding or by transmission of virus. *Aphis craccivora* Koch causes severe damage to a broad spectrum of crops. Due to the development of resistance to insecticides and their adverse effects on humans and the environment, alternative safer aphid control methods are required.

Extracts from seed kernels of the neem tree (*Azadirachta indica* A. Juss.) and *Melia azedarach* L.

Corresponding author: NZD.

contain several active principals (Warthen, 1979; Kraus et al., 1981). The biological activity of neem extracts on aphids has been studied by several workers (Goyal et al., 1971; Siddig, 1981; Dimetry and Schmidt, 1992; Dimetry and El-Hawary, 1995).

The possibility of increasing the efficacy of plant extracts by addition of different synergists such as sesame oil or piperonyl butoxide has been examined (Dove, 1947). A few authors have also discussed the possibility of a synergistic effect on neem seed extracts against different pests (Lange and Schmutterer, 1982; Lange, 1984; Schauer, 1984; Dimetry and Schmidt, 1991).

In this paper, we report the results of bioassays to ascertain whether the effectiveness of different commercial neem formulations could be improved by addition of different synergists, namely ethyl oleate, sesame oil and dimethyl sulphoxide (DMSO).

MATERIALS AND METHODS

Bioassay

A stock culture of *Aphis craccivora* Koch was maintained on broad bean (*Vicia faba*) seedlings under laboratory conditions at $20 \pm 5^\circ\text{C}$, $65 \pm 5\%$ RH with a 12:12 (L:D) h photoperiod. Bioassays were conducted under the same conditions.

Different concentrations of the commercial neem formulations Neem Azal-T (5% azadirachtin) and Neem Azal-T/S (1% azadirachtin) (Trifolio-MG mbH, Germany) were prepared using 0.01% Tween® (80) (El Nasr Pharmaceutical Chemicals Co.) as emulsifier and distilled water as solvent. In all experiments, aphids were put on cultivated fresh, 7-day-old broad bean seedlings enclosed individually in glass cylinders, 10 cm diameter, 22-cm long, the tops of which were covered with muslin held in place with rubber bands.

In experiments on additives, 0.6% aqueous solutions of ethyl oleate, sesame oil, DMSO and CaCO_3 were prepared. Ethyl oleate and sesame oil were emulsified with 0.01% Tween. Different formulations were prepared in the ratio of 1 part neem formulation extract to 10 parts of additive.

Fifteen newly formed virgin apterae of *A. craccivora* were placed on a bean plant and the plant sprayed with 5 ml of each concentration of Neem Azal-T or Neem Azal-T/S. An equal number of pots containing untreated plants were sprayed with distilled water plus 0.01% Tween and served as controls. The experiment was replicated five times.

Data on fecundity and longevity of the adult aphid were recorded daily and analysed statistically using F-test according to Hayslett (1970).

Toxicity tests

Five newly formed apterous adults of *A. craccivora* were placed on broad bean plants and treated with different concentrations of the Neem Azal-T or Neem Azal-T/S. Another group of five newly emerged apterous adults was placed on bean plants treated with 0.06% Neem Azal-T or Neem Azal-

T/S containing the different additives mentioned before at a concentration of 0.6%. A third group of five adults was placed on plants treated with water and Tween and served as a control. Five replicates were tested for each concentration. Adult mortalities in all cases were recorded at 24, 48, 72 and 96 h after treatment. All data were corrected according to Abbott (1925).

RESULTS AND DISCUSSION

Effects of neem seed extracts without additives

Treatment of *Aphis craccivora* with Neem Azal-T or Neem Azal-T/S significantly reduced aphid fecundity. Control females produced about 13 and 9 times more nymphs during their reproductive period and their adults survived about 9 and 7.5 days longer than those treated with 0.06% Neem Azal-T and Neem Azal-T/S, respectively (Table 1). The aphid's reproductive period decreased significantly at all concentrations tested for both extracts compared with control. There was no significant difference between the two formulations in terms of their effect on fecundity and longevity of *A. craccivora* (Table 1).

These results are in agreement with data obtained by Dimetry and El-Hawary (1995) using Neem Azal-F against *A. craccivora*, Dimetry and Schmidt (1992) and Schauer (1984) using different neem and china-berry extracts against *Aphis fabae* and *Acyrtosiphon pisum*, respectively.

It can be concluded that both Neem Azal formulations serve as contact larviposition deterrents for *A. craccivora* since fecundity decreased significantly in comparison with the control. The two extracts also possess an antifeedant effect, which is consistent with the works of Islam (1984), Schmutterer (1984) and Tanzubil (1987) who investigated the aphicidal and antifeedant activity of neem.

Effects of neem seed extracts with additives

Table 2 shows that the efficacy of Neem Azal-T/S was increased significantly when additives ethyl oleate or sesame oil were used, but addition of DMSO or CaCO_3 had no significant effect on fecundity in comparison with using the extract alone. There was, however, a significant effect on the longevity of *A. craccivora* when the extract was used with DMSO as an additive in comparison with the extract alone.

Table 1. Effect of different concentrations of Neem Azal-T and Neem Azal-T/S on the biology of *Aphis craccivora*

Extract concentration (%)	Neem Azal-T			Neem Azal-T/S		
	Average period (days) \pm SE			Average period (days) \pm SE		
	Fecundity ¹ \pm SE	Reproductive period	Longevity	Fecundity \pm SE	Reproductive period	Longevity ¹
0.5	1.8 \pm 0.7	1.3 \pm 0.6	2.6 \pm 0.5	1.5 \pm 0.6	0.8 \pm 0.3	2.0 \pm 0.4
0.25	2.4 \pm 1.0	1.4 \pm 0.5	2.6 \pm 0.9	2.1 \pm 0.7	0.7 \pm 0.3	2.2 \pm 0.6
0.125	3.8 \pm 1.0	1.8 \pm 0.5	3.3 \pm 6.2	5.4 \pm 1.1	1.8 \pm 0.4	3.7 \pm 0.9
0.06	6.3 \pm 2.3	3.1 \pm 1.1	4.9 \pm 1.4	9.0 \pm 1.9	3.7 \pm 1.1	6.2 \pm 1.1
Control	84.1 \pm 5.6	12.8 \pm 1.0	13.6 \pm 1.0	84.1 \pm 5.6	12.8 \pm 1.0	13.6 \pm 1.0
F-value	151.051**	40.872**	26.789**	116.799**	57.829**	40.807**
L.S.D. 5%	8.631	2.257	2.600	10.784	2.185	2.420
L.S.D. 1%	11.483	3.001	3.459	14.412	2.920	3.235

**Significant at 0.01% level.

¹Fecundity = number of young laid per female.Table 2. Effect of different additives¹ as synergists to Neem Azal-T and Neem Azal-T/S against *Aphis craccivora*

Additives ¹	Neem Azal-T			Neem Azal-T/S		
	Average period (days) \pm SE			Average period (days) \pm SE		
	Fecundity ² \pm SE	Reproductive period	Longevity	Fecundity ² \pm SE	Reproductive period	Longevity
Ethyl oleate	1.2 \pm 0.8	0.8 \pm 0.5	1.6 \pm 0.4	2.2 \pm 1.4	1.0 \pm 0.5	2.2 \pm 0.7
Sesame oil	3.2 \pm 1.4	1.2 \pm 0.4	1.6 \pm 0.4	3.0 \pm 1.8	1.0 \pm 0.5	2.2 \pm 0.8
Dimethyl sulphoxide	2.8 \pm 1.5	1.2 \pm 0.6	2.0 \pm 0.6	5.6 \pm 2.6	1.5 \pm 0.6	2.2 \pm 0.7
CaCO ₃	5.1 \pm 0.9	3.0 \pm 0.3	4.3 \pm 0.3	8.3 \pm 1.2	2.8 \pm 0.5	4.7 \pm 0.6
Control (no additives)	6.3 \pm 2.3	3.1 \pm 1.1	4.9 \pm 1.4	9.0 \pm 1.9	3.7 \pm 1.1	6.2 \pm 1.1
F-value	0.995	1.345	2.014	2.810*	3.370*	5.835**
L.S.D. 5%	-	-	-	5.326	1.898	2.239
L.S.D. 1%	-	-	-	7.208	2.568	3.031

*Significant at 0.05% level.

**Significant at 0.01% level.

¹Additive as a 0.6% aqueous solution. Ethyl oleate and sesame oil emulsified with 0.01% Tween®. Ratio of 1:10 extract: additive was used.²Fecundity = number of young laid per female.

On the other hand, none of the additives showed any synergistic effect on Neem Azal-T with respect to any of the measured biological parameters of *A. craccivora* adults.

It could be concluded that the effect of additives on Neem Azal-T/S is more pronounced than on Neem Azal-T. The synergistic effects most probably result from improved penetration of the active material into the treated plants.

The present findings are in agreement with those of Stark and Walter (1995) who indicated

that neem oil and other oils increase the efficacy of neem-based insecticides.

Toxicity effects without additives

Table 3 shows that both Neem Azal-T and Neem Azal-T/S caused a significant increase in the mortality of *A. craccivora* adults 96 h after treatment in a concentration-dependent manner. Dimetry and El-Hawary's (1995) studies with Neem Azal-F corroborate the present findings.

Table 3. Effect of different concentrations of Neem Azal-T and Neem Azal-T/S on the percentage mortality of *Aphis craccivora*

Extracts	% concentration	Corrected mortality (%) at				Cumulative
		24 h	48 h	72 h	96 h	
Neem Azal-T	0.5	53.3	20.0	20.0	0.0	93.3
	0.25	50.0	40.0	0.0	0.0	90.0
	0.125	40.0	30.0	15.0	5.0	90.0
	0.060	66.7	13.3	6.7	0.0	86.7
Neem Azal-T/S	0.5	60.0	30.0	0.0	0.0	90.0
	0.25	33.3	33.3	13.3	6.7	86.7
	0.125	65.0	15.0	0.0	0.0	80.0
	0.060	45.0	0.0	15.0	10.0	70.0

Table 4. Effect of different additives⁺ on the toxicity of Neem Azal-T and Neem Azal-T/S to *Aphis craccivora*

Extracts	Additives	Corrected mortality (%) at				Cumulative
		24 h	48 h	72 h	96 h	
Neem Azal-T	Ethyl oleate	68.0	8.0	12.0	8.0	96.0
	Sesame oil	70.0	20.0	0.0	5.0	95.0
	Dimethyl sulphoxide	60.0	20.0	0.0	14.0	94.0
	CaCO ₃	56.7	23.0	0.0	6.7	86.3
	Control	66.7	13.3	6.7	0.0	86.7
Neem Azal-T/S	Ethyl oleate	60.0	10.0	15.0	10.0	95.0
	Sesame oil	64.0	0.0	26.0	0.0	90.0
	Dimethyl sulphoxide	64.0	26.0	0.0	0.0	90.0
	CaCO ₃	0.0	20.0	18.0	30.0	68.0
	Control	40.0	0.0	15.0	15.0	70.0

⁺ Additive as a 0.6% aqueous solution. Ethyl oleate and sesame oil emulsified with 0.01% Tween. Ratio of 1:10 extract: additive was used.

Toxicity effects with additives

The insecticidal efficacy of either Neem Azal-T or Neem Azal-T/S increased significantly by addition of different additives (Table 4). Addition of ethyl oleate, sesame oil or DMSO improved the effects of both Neem Azal-T or Neem Azal-T/S extracts in achieving higher mortalities of *A. craccivora* adults. On the other hand, addition of CaCO₃ showed a negative correlation as the percentage mortality of the adults was nearly the same in comparison with the extracts alone.

The present findings are in agreement with those of Schauer (1984) who found that addition of sesame oil to a tert-methyl butyl ether extract of neem resulted in higher mortality of *Acyrtosiphon pisum* and *Aphis fabae* than did the same extract without sesame oil.

Additives may have a synergistic effect which increase the efficacy of an extract. This is

ascertained by Metcalf (1967) who found that the synergists inhibit the enzymes responsible for toxicant degradation. Othaki et al. (1968) and Othaki and Williams (1970) stated that the insect body contains enzymes for the degradation of hormones like the moulting hormone which may be a mode of action of neem formulation. However, Walker and Thompson (1973) found that simultaneous application of moulting hormone (MH) and juvenile hormone (JH) caused an increase in MH activity. In this case it is significant that azadirachtin is said to act like an MH mimic (Schmutterer and Rembold, 1980).

CONCLUSION

Both Neem Azal-T and Neem Azal-T/S had an aphicidal effect against *A. craccivora* and significantly reduced fecundity and longevity of adult aphids. Addition of synergists like ethyl

oleate and sesame oil to neem extracts improved the efficacy, and thereby increased mortalities and decreased the fecundity of adults significantly.

REFERENCES

- Abbott W. S. (1925) A method of computing the effectiveness of an insecticide. *J. econ. Entomol.* 18, 265-267.
- Dimetry N. Z. and El-Hawary F. M. (1995) Neem Azal-F as an inhibitor of growth and reproduction in the cowpea aphid *Aphis craccivora* Koch. *J. appl. Entomol.* 119, 67-71.
- Dimetry N. Z. and Schmidt G. H. (1991) Improvement of methanol extract of *Melia azedarach* by some additives against *Aphis fabae* Scop. *Boll. Zool. Agr. Bahic*, Ser II, 23, 143-151.
- Dimetry N. Z. and Schmidt G. H. (1992) Efficacy of Neem Azal-S and Margosan-O against the bean aphid *Aphis fabae* Scop. *Anz. Schadlingskde, Pflanzenschutz, Umweltschutz* 65, 75-79.
- Dove W. E. (1947) Piperonyl butoxide, a new safe insecticide for household and field. *Am. J. Trop. Med.* 27, 339-345.
- Goyal R. S., Gulati K. C., Sarup P., Kidwai M. A. and Singh D. S. (1971) Biological activity of various alcohol extractives and isolates of neem (*Azadirachta indica*) seed cake against *Rhopalosiphum nymphaeae* (Linn.) and *Schistocerca gregaria* Forsk. *Indian J. Entomol.* 33, 67-71.
- Hayslett H. T. (1970) *Statistics Made Simple*. W.H. Allen, London.
- Islam B. N. (1984) Pesticidal action of neem and certain indigenous plants and weeds of Bangladesh. *Proc. 2nd Int. Neem Conf.* (Rauischholzhausen, 1983), pp. 263-290.
- Kraus W., Cramer R., Bokel M. and Sawitzki G. (1981) New insect antifeedant from *Azadirachta indica* and *Melia azedarach*. *Proc. 1st Int. Neem Conf.* (Rottach-Egern, 1980), pp. 53-62.
- Lange W. (1984) Piperonyl butoxide: Synergistic effects on different neem seed extracts and influence on degradation of an enriched extract by ultra-violet light. *Proc. 2nd Int. Neem Conf.*, Rauischholzhausen, 1983, pp. 129-140.
- Lange W. and Schmutterer H. (1982) Versuche mit synergisten Zur Steigerung der metamorphose Storenden Wirkung eines methanolischen Rohextraktes aus Samen des Neembaumes (*Azadirachta indica*). *Z. Pfl.-Krankh., Pfl.-Schutz.* 89, 258-265.
- Metcalf R. L. (1967) Mode of action of insecticide synergists. *Annu. Rev. Entomol.* 12, 229-256.
- Othaki T., Milkma R. D. and Williams C. M. (1968) Dynamics of ecdysone secretion and action in the flesh fly, *Sarcophaga peregrina*. *Biol. Bull. mar. biol. Lab.*; Woods Hole 135, 322-334.
- Othaki T. and Williams C. M. (1970) Inactivation of β -ecdysone and cyasterone by larvae of the flesh fly, *Sarcophaga peregrina* and pupae of the silkworm *Sesamia cynthia*. *Biol. Bull. mar. biol. Lab.*, Woods Hole 138, 326-333.
- Schauer M. (1984) Effects of variously formulated neem seed extracts on *Acyrtosiphon pisum* and *Aphis fabae*. *Proc. 2nd Int. Neem Conf.* (Rauischholzhausen, 1983), pp. 141-150.
- Schmutterer H. (1984) Neem research in the Federal Republic of Germany since the first international Neem Conf. *Proc. 2nd Int. Neem Conf.* (Rauischholzhausen, 1983), pp. 21-30.
- Schmutterer H. and Rembold H. (1980) Zur Wirkung einiger Reinfractionen aus Samen von *Azadirachta indica* auf Frassaktivität und Metamorphose von *Epilachna varivestis* (Col., Coccinellidae). *Z. angew. Entomol.* 89, 179-188.
- Siddig A. S. (1981) Evaluation of neem (*Azadirachta indica* A. Juss.) as protectant against some major pests in the Sudan. *Annual Report Entomology Unit, Shambat Research Station, Sudan*, 8 pp.
- Stark J. D. and Walter J. F. (1995) Neem oil and neem oil components affect the efficacy of commercial neem insecticides. *J. Agric. Food Chem.* 43, 507-512.
- Tanzubil P. B. (1987) The use of neem products in controlling the cowpea weevil, *Callosobruchus maculatus*. *Proc. 3rd Int. Neem Conf.* (Nairobi, 1986), pp. 517-523.
- Walker W. F. and Thompson M. J. (1973) 22,25-Bisdeoxyecdysone pathological effects on the Mexican bean beetle and synergism with juvenile hormone compounds. *J. econ. Entomol.* 66, 64-67.
- Warthen J. D. Jr. (1979) *Azadirachta indica*: A source of insect feeding inhibitor and growth regulators. *US Dep. Agric., Res. Results ARR-NE-4*.

THIS PAGE BLANK (USPTO)